Information pest: *Grapevine red blotch-associated virus*

Grapevine red blotch-associated virus (GRBaV) is the latest addition to the list of more than 75 graft-transmissible agents that have been identified in grapevines. Based on the wide host and geographic distribution of GRBaV and the fact that the virus is transmitted by grafting, it is likely that spread primarily occurs through propagation material.

It was described for the first time on Cabernet Sauvignon in Napa Valley in 2008. Infected vines have been identified in California, New York, Virginia, Maryland.

Symptoms of the disease are very similar to leafroll disease. Like leafroll, leaves turn red in early fall primarily at the base of the shoots. Unlike leafroll, vines with red blotch disease show pink/red veins on the leaf undersides and no rolling.

Introduction

The SYBR GRBaV set has been developed and optimized by Qualiplante, based on Krenz et al., 2014. The *coat protein* gene (*CP* gene) was used to design primer pair.
A poster "Comparison of two PCR technics used in GRBaV detection" (available on request) has been presented during the 18th Conference of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine in September 2015 at Ankara (Turkey) showing the performance of this SYBR GRBaV set.
This product should be used only for research purposes.

Intended use

The SYBR PCR set is validated for the detection of *Grapevine red blotch-associated virus* by Real-Time PCR (SYBR-Green® technology).

The SYBR-Green® technology allows to confirm that a sample generating an amplification signal is produced only by nucleic acids of the pathogen of interest, by interpretating the melting peak.

Suitable tissues are grapevine leaves, in particular leaf petioles, and bark scrapings from dormant canes.

Set format and content

Two sets are available for 24 and 96 tests.

Article N°	Product name
7GRBaVS2	SYBR GRBaV set 24
7GRBaVS9	SYBR GRBaV set 96

Content	set 24	set 96
Direct Master Mix	24 tests 7GRBaVS2-DM-	2x48 tests 7GRBaVS9-DM-
Positive Control	3 tests 7GRBaVS2-PC-	8 tests 7GRBaVS9-PC-
Negative Control	3 tests 7GRBaVS2-NC-	8 tests 7GRBaVS9-NC-

Storage conditions

This set can be shipped at room temperature but upon receipt it should be stored immediately at the recommended storage temperature: **from -30 ° C to -10 ° C**.
Avoid prolonged exposure to light and repeated freeze and thaw cycles.

Shelf life

If the set is correctly stored, at constant-temperature freezer, its performance is guaranteed until the expiration date indicated on the tubes label.

Materials and equipment (not provided)

- DNA extraction tools and reagents
- Nuclease-free filter tips and micropipettes
- Optical grade nuclease-free tubes/plate
- Disposable latex or vinyl gloves
- Thermal cycler for Real-Time PCR with filters calibrated for SYBR-Green®

Nucleic acids extraction

Extract DNA from samples according to your usual protocol. Upon request, Qualiplante can recommend you an extraction method.

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Reaction set-up

a) Slowly thaw Direct Master Mix by placing it on ice or store at +4°C.
b) Shake briefly Direct Master Mix and spin down the liquid.
c) Add 17 µl of Direct Master Mix (without DNA template) to each PCR tubes or wells of an optical-grade PCR plate.
d) Add 3 µl of DNA template to the Direct Master Mix. Do not forget to prepare a PCR tube or well of an optical-grade PCR plate for the Positive Control and the Negative Control.

Components	Volume/PCR tube or well
DNA template or Positive control or Negative control	3 µl
Direct Master Mix	17 µl
Total Volume / PCR tube or well	20 µl

In order to confirm the absence of any reagent's contamination, we strongly recommend including a no-template control (e.g. DEPC water) in the assay.

Run and thermal cycling

a) Seal carefully the PCR tubes or PCR plate. Centrifuge briefly to collect components at the bottom of the PCR tubes or wells of the plate. Protect from light before thermocycling.
b) Load the PCR tubes or plate into the thermal-cycler and follow the thermal cycling below:

Steps	Temp (°C)	Time	Cycle(s)
UDG activation	50°C	2 min	1
Enzyme activation	95°C	2 min	1
Denaturation	95°C	15 sec	40
Annealing/Elongation	60°C	1 min	
Melt temperature	Follow the instructions of your thermal cycler		

Results analysis

The reaction for GRBaV will generate a specific SYBR®-labeled amplification curve and a specific melting-curve.

Fig.1: Example of amplification curves.

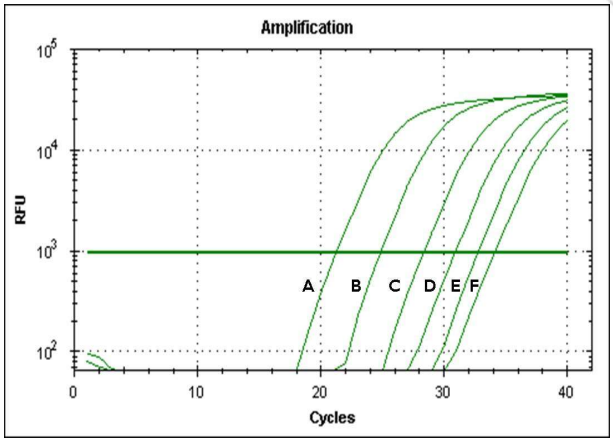

fig.1 shows amplification curves associated to a GRBaV infected sample diluted at different level or Positive Control of the set. Dilutions **A:** 1:10 - **B:** 1:100 - **C:** 1:1,000 - **D:** 1:5,000 - **E:** 1:10,000 - **F:** 1:50,000.

Fig.2: Example of melting curves.

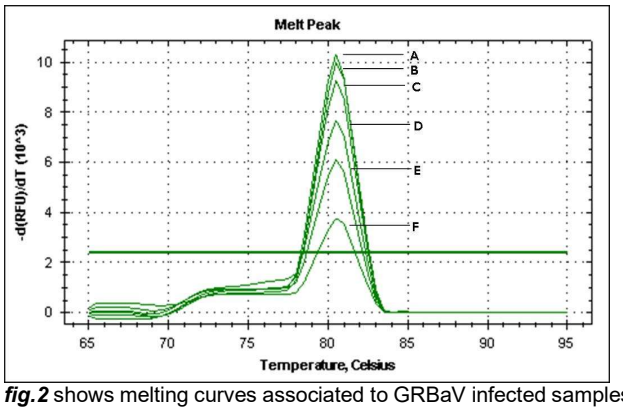

fig.2 shows melting curves associated to GRBaV infected samples diluted at different level or Positive control of the set (Tm=81,5°C on Biorad CFX96 machine). Dilutions **A:** 1:10 - **B:** 1:100 - **C:** 1:1,000 - **D:** 1:5,000 - **E:** 1:10,000 - **F:** 1:50,000.

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ANALYSIS VALIDATION AND RESULTS INTERPRETATION

For a correct interpretation of results, always:

- check if results of Positive Control and Negative Control pass,
- combine amplification curves analysis with melting curves analysis,
- confirm that the samples melting temperatures match the Positive Control melting temperature.

Step 1: Check the Ct values of Positive Control and Negative Control

Well	Ct	Interpretation
Positive Control	38 or less	Go to step 2 and 3
	Above 38 or no Ct	Fail*
Negative Control	No Ct	Go to step 2 and 3
	Less than 35	Fail**

* Repeat the assay, ensuring that steps of the user guide are carefully performed
** The mix or the Negative Control was contaminated with GLRaV-1 nucleic acids. Repeat the assays after identifying and removing the potential source of contamination

Step 2: Check Ct value in the samples well

Well	Ct	Interpretation
Sample	38 or less	Go to step 3
	More than 38 or no Ct	Negative

Step 3: See melting temperature

Well	Tm	Interpretation
Sample	Tm differs no more than ± 1°C from Tm of Positive Control	Positive
	Tm differs more than ± 1°C from Tm of Positive Control	Negative

Special handling instructions

This set was designed to be used by laboratory staff trained to follow the usual molecular biology precautions. Always perform the tests in a nuclease-free work environment. Always wear gloves when handling samples containing DNA/RNA and the components of the set. Do not touch any set components with an ungloved hand. Use appropriate laboratory disposable parts. Use nuclease-free tubes and filter tips to avoid degradation and cross-contamination. Do not use components from sets with different batch numbers in the same test procedure. Do not interchange reagents with other sets. To avoid cross-contamination, use separate rooms for (a) nucleic acids extraction, (b) preparation of the Master Mix and (c) amplification. To avoid cross-contamination and obtain reliable results, it is essential to strictly follow the protocol in this manual. Avoid unnecessary freeze-thaw cycles of the set components. Do not use reagents after their expiration date.

Troubleshooting

Post-PCR data analysis shows no amplification, or amplification plots look grossly abnormal:

Possible causes	Corrective actions
Evaporation of the sample due to inadequate sealing of the plate	Repeat the test using the appropriate tools to seal correctly the plate
Consumables are not appropriate for the method	Repeat the test using consumables recommended by the thermal cycler supplier
The quality of nucleic acid extracted is low	Repeat the extraction step. Ensure that the method of extraction has been performed correctly. In any doubt, contact us
Abnormal amplification	Centrifuge the plate briefly to spin down the contents and eliminate any air bubbles

No amplification reaction is observed in the positive control well, while other samples are positive:

Possible causes	Corrective actions
The positive control provided with the set was not added into the reaction well	Repeat the test. If the problem persists, contact us

An amplification plot is observed in the negative control well:

Possible causes	Corrective actions
Contamination of the negative control or the Master Mix with target-positive nucleic acid	Repeat the test by applying appropriate quality procedures to prevent contamination. Seal the plate correctly

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